Science Advances

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advances.sciencemag.org/cgi/content/full/4/5/eaat1293/DC1

# Supplementary Materials for

## Dynamical features in fetal and postnatal zinc-copper metabolic cycles predict the emergence of autism spectrum disorder

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Published 30 May 2018, *Sci. Adv.* **4**, eaat1293 (2018)
DOI: 10.1126/sciadv.aat1293

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## Supplementary Materials and Methods

### Study Populations

We undertook this study in four geographically distinct populations. An important consideration for our discovery analysis was to apply control over genetic factors, which we achieved by comparing individuals with ASD with their discordant twin siblings and non-ASD twins in the Roots of Autism and ADHD Twin Study (RATSS). However, after discovery, we considered it important to extend our findings to non-twin siblings and unrelated participants. We also preferred to include participants from different geographic regions, rather than undertake replication in a single study. To achieve this aim, our primary criteria for inclusion were that children enrolled in established studies with an ASD diagnosis that required treatment by a medical professional, were able to provide naturally shed deciduous teeth, and had no known neurodevelopmental disorder or genetic syndromes. Since our analysis does not include cross-population comparisons of diagnostic indices, we included children diagnosed with any validated clinical diagnostic tests for ASD. Our study has 75 discovery participants, and an additional 118 participants for our replication analysis (Table 1).

**Roots of Autism and ADHD Twin Study (RATSS).** Description of the RATSS for projects using teeth has been reported before (*1*). Participating twins in this study are part of RATSS recruited between 2011 and 2016 (*24*). The study was approved by the Swedish Regional Ethical Review Board and all participants gave written informed consent. Potential twin participants for the RATSS are identified through nationwide registries, including the Child and Adolescent Twin Study in Sweden (CATSS) (*35*), a population-based study of all twins born in Sweden since 1992 in which all twins are screened at age nine using the Autism, Tics, ADHD and other

Comorbidities Inventory (A-TAC). Participants are identified through linking the Swedish Twin Registry to other National registries such as the Swedish National Patient Register, and regional clinical registers in Stockholm County (Child and Adolescent Psychiatry ["Pastill"], Habilitation & Health Centers) that include ICD-10 diagnostic information (*36, 37*). Finally, potential participants are also identified through Swedish societies for neurodevelopmental disorders (NDDs) as well as advertisements and summons in the media. Even though the recruitment is done through different routes, >80% of the twins in RATSS are present in the Swedish twin registries.

Twin pairs were recruited into the RATSS either based on discordance for ASD (>2 points differences on the A-TAC autism subscale equaling ~1 SD); concordance for ASD (both twins reaching cut-off on the A-TAC autism scale); or concordance for no NDD (both twins under cut-offs for all NDD subscale on the A-TAC). For other sources of recruitment, the twins are invited if at least one twin has an ICD-10 diagnosis of autism (F84.0), Asperger syndrome (F84.5), or atypical autism/pervasive developmental disorder not otherwise specified (PDD-NOS) (F84.1, F84.8, F84.9), or a Diagnostic and Statistical Manual of Mental Disorders Fifth Edition (DSM-5) diagnosis of ASD (either parent- or registry reported). All potential participants undergo a telephone interview by a research nurse checking eligibility before the invitation for assessment in RATSS. Zygosity was determined by genotyping of saliva, or whole-blood derived DNA using standard methods. The genotyping was done using Infinium Human-CoreExome chip (Illumina Inc. USA). The estimating identity by descent was analyzed using the PLINK software (v1.07) (*38*) after quality control and removal of SNPs with minor allele frequency less than 0.05 within the samples. All pairs of DNA samples showing $\hat{\pi}$ >=0.99 were considered as

monozygotic pairs. For few pairs, a short tandem repeat kit (Promega Powerplex 21) was used to determine the zygosity.

***Ascertainment of ASD Cases and Controls:*** Medical history and sociodemographic information of the families were collected. ASD was diagnosed according to DSM-5 criteria based on clinical experts consensus and corroborated by results from the Autism Diagnostic Interview – Revised (ADI-R) (*39*) and the Autism Diagnostic Observation Schedule Second Edition (ADOS-2) (*40*). Clinical severity of ASD symptoms was determined by ADOS comparison scores, and autistic traits were measured by parent reported Social Responsiveness Scale-2 (SRS-2) (*41*) total raw scores. General cognitive ability was assessed using the Wechsler Intelligence Scales for Children or Adults (Fourth Editions) or the Leiter Scales and the Peabody Picture Vocabulary Test (Third Edition) in cases of low verbal abilities (*42-44*). Our study included both identical/monozygotic twins and fraternal or dizygotic. There were 34 MZ twins (17 pairs). Additional details have been published elsewhere (*1*).

***Tooth Collection and Storage:*** Parents/guardians collected the naturally shed deciduous teeth at home. Teeth were brought to the study team in person and stored at room temperature until analysis.

**Avon Longitudinal Study of Parents and Children (ALSPAC)**

ALSPAC is a longitudinal cohort study which recruited 14,541 pregnant women resident in Avon, UK with expected dates of delivery 1st April 1991 to 31st December 1992. 14,541 is the initial number of pregnancies for which the mother enrolled in the ALSPAC study and had either

returned at least one questionnaire or attended a "Children in Focus" clinic by 19/07/99. Of these initial pregnancies, there were a total of 14,676 fetuses, resulting in 14,062 live births and 13,988 children who were alive at 1 year of age. When the oldest children were approximately 7 years of age, an attempt was made to bolster the initial sample with eligible cases who had failed to join the study originally. The number of new pregnancies not in the initial sample (known as Phase I enrolment) that are currently represented on the built files and reflecting enrolment status at the age of 18 is 706 (452 and 254 recruited during Phases II and III respectively), resulting in an additional 713 children being enrolled. The phases of enrolment are described in detail (*25*). The total sample size for analyses using any data collected after the age of seven is therefore 15,247 pregnancies, resulting in 15,458 fetuses. Of this total sample of 15,458 fetuses, 14,775 were live births and 14,701 were alive at 1 year of age (study website contains details of all data available: http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/) (*25*, *45*). Regular data collection is ongoing since September 6th 1990. Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees (listed at http://www.bristol.ac.uk/alspac/researchers/research-ethics/). All participants provided written informed consent.

***Ascertainment of ASD Cases and Controls:*** The identification of ASD cases in ALSPAC is described in detail elsewhere (*46*). Briefly, information of ASD diagnosis was obtained from UK National Health Service (NHS) and education sources. The use of multiple sources was implemented to ensure as complete an ascertainment as possible (*47*). All ALSPAC cohort members who had a World Health Organization's International Statistical Classification of Diseases and Related Health Problems, Tenth Revision (ICD-10) diagnosis relating to any form

of developmental delay for the period 1991 to 2003 inclusive, or those who were identified in the NHS Child Health computer system as having special educational needs during the same time period were identified, and their health records were obtained from the NHS.

All the NHS Trusts in the ALSPAC area have specialist autism teams in children's services, trained to use standardized assessment tools including the Diagnostic Interview for Social and Communication Disorders (*48*); the Autism Diagnostic Observation Schedule (*49*), and the Asperger Syndrome Diagnostic Interview (*50*). A team of three experienced researchers searched NHS hospital medical records (in-patient and outpatient) and community child health records (including child development team records) to identify children who had a diagnosis of ASD made after a multidisciplinary assessment. The researchers searched for ICD-10 diagnoses according to a structured proforma, based on information that was available in the records written by the multiprofessional team involved in the care of the child. A board-certified pediatrician with 30 years of clinical experience reviewed all information collected from the notes and confirmed that the diagnostic information was consistent with ICD-10 criteria.

In addition, the Department of Education for England Pupil Level Annual Schools Census (PLASC) dataset which included data on children attending state schools who were recorded as having some form of special educational needs was searched for ALSPAC children with ASD listed as either a primary or a secondary concern.

***Tooth Collection and Storage:*** Teeth were collected when participants were 5-7 years old. Mothers were asked for any teeth shed naturally or extracted by a dentist, without cavities or

fillings, to be sent to the ALSPAC team. Mothers were asked to keep teeth at room temperature and to indicate the date when the tooth was shed. Approximately 70% of those contacted provided teeth, and those were kept at -20ºC. For this study, teeth samples from 25 ASD cases and 25 healthy controls with no documented developmental, learning or psychiatric disorder were obtained for the present study. Cases and controls were matched for sex and birth weight.

**Seaver Autism Center, New York, USA**

The Seaver Autism Center is located at the Icahn School of Medicine at Mount Sinai in New York City and serves a diverse and complex patient population. The Seaver Center has longstanding community ties and receives approximately 600 new autism referrals annually for research and/or clinical services. Ethical approval for the study was obtained from Mount Sinai School of Medicine research ethics committee. All participants and/or their parents provided written informed consent.

***Ascertainment of ASD Cases and Controls:*** In 2016 we contacted all families in ongoing studies and services at the Seaver Center that (1) had a child with ASD as well as an additional child without a diagnosis of ASD, and (2) the case and sibling where of teeth-shedding age (ages 5-10). Informed consent was obtained from all parents or legal guardians. ASD diagnosis was confirmed through a gold-standard evaluation including the Autism Diagnostic Observation Schedule, Second Edition (ADOS-2) (*51*), and the Autism Diagnostic Interview-Revised (ADI-R) (*52*) and a clinical evaluation with a board-certified child and adolescent psychiatrist or licensed clinical psychologist to assess DSM-5 (*53*) criteria for ASD.

***Tooth Collection and Storage:*** Caregivers were provided with an individual collection kit and a corresponding questionnaire for each tooth. The questionnaire included the following multiple choice questions: (1) How was the tooth obtained? Response options: Lose and fell out naturally, removed by a dentist, unknown, other; (2) How was the tooth stored? Response options: Dry, in a plastic bag, in water, in another liquid, unknown, other. Parents were asked to specify when "other" was selected. Demographic information was provided by caregivers for all probands and siblings. These included information on gestational age, birth weight, significant perinatal medical history, and psychiatric diagnoses. Teeth samples from 10 ASD cases and 8 of their siblings with no documented developmental, learning or psychiatric disorder were obtained for the present study. This sample was predominantly white American (77%).

**University of Texas Health Sciences Center, Houston, Texas, USA**

In January 2011, deciduous tooth collection was commenced in a national sample of families enrolled in the Interactive Autism Network (IAN). The IAN network is the nation's largest online autism research forum where over 43,000 families complete comprehensive surveys and participate in various research studies. Inclusion criteria for IAN participants are parents with children under 18 years of age who have been diagnosed with ASD by a health professional. Twenty-five cases and 25 gender-matched controls were selected for inclusion in this study.

***Ascertainment of ASD Cases and Controls:*** The diagnosis of ASD in the IAN database has been clinically validated in a subsample of participants[89] as well as verified by a review of parent- and professional-provided medical records[90]. This work reports 98% concordance of validated ASD diagnoses with parental self-report, thus supporting the viability of the centralized database

recruitment model. Notwithstanding, to verify diagnosis in our non-IAN recruits, a certified diagnostician recently conducted an ADIR interview by phone in a random subset of our participants. We found that there was a 100% concordance between parent-reported and diagnostician assessed ASD. In the current study, we exclude the use of teeth from children whose mothers report having children with various other medical or neurological conditions including ADHD, and retain only those with ASD or those that are typically developing controls with no reported medical, emotional or psychological comorbidities.

We have recruited unrelated population controls through television news spots, print media advertisement, as well as a wide consortium of community-based partners that include several children's dental and well-child medical clinics. Similar to case mothers, control mothers who choose to participate are asked to submit one or more teeth from each of her children. While a potential source of controls might be obtained from families who are friends of case families, this is not recommended in case-control studies due to unknown inherent confounding biases.[88]

***Tooth Collection and Storage:*** Potential participants contact our research coordinator who then screens them for eligibility. Each mother must be the biological mother of the child whose deciduous teeth will be provided. If eligible, families receive a study packet in the mail consisting of a consent form, tooth envelope, return envelope and a survey. The survey asks information about family demographics, medical history, pre and postnatal care and brief exposure histories. For families with more than one child, separate surveys and shipping packets are mailed.

When the material is returned (we currently have a 90 % return rate from eligible families requesting a packet), each survey and tooth is then given a unique study identification number that links surveys to teeth. Both teeth and survey data are entered into our database and stored in locked file cabinets until they are selected for analysis. For both case and control teeth, we utilize canine, molar and incisor teeth without cavities or fillings. We matched the case and controls in this study by gender and approximate age (within a 5-year range). Teeth are stored at room temperature until analysis.

**Bioequivalence Testing**

For bioequivalence testing of RQA/CRQA measures of control subjects across populations, we extended standard equivalence testing methods following the principles of confidence interval inclusion[14]. These provide confidence intervals (CIs) on the comparative ratios of population means for a given measure, with equivalence inferred from containment of family-wise CIs within a pre-defined equivalence bound; here, as per FDA guidelines (FDA-CDER, 2003) (*54*), ranging from 0.80 - 1.25. Asymmetric confidence intervals were constructed by back-transforming confidence intervals from Dunnett's test (with family-wise 95% confidence coefficient, with the discovery cohort as reference) on the difference of logs constructed using least square means adjusted for covariates (here, sex) in a general linear model of each log-transformed measure. Analyses were conducted using PROC GLM in SAS (v.9.4).

**Statistical Models**

Linear mixed models were used to test for global and population-specific effects of ASD diagnosis on dynamical features measured with RQA, e.g. mean diagonal length (MDL),

entropy, determinism. These models followed the equation: $y = X\beta + Zu + \varepsilon$, where y is an RQA feature (e.g., MDL), Xβ reflects fixed effects and associated observations, Zu reflects a design matrix and associated random effect to account for the relatedness, and therefore potential non-independence, of subjects (twins, siblings, or unrelated), with ε as a vector of random errors. The primary fixed effects relating to our study question were ASD (diagnosis as case or control), to test for global (across study) differences in ASD, and an ASD*Study interaction, to test for population-specific differences in ASD, with sex included as a covariate. Additional covariate variables, including zygosity of twins, diagnosed comorbidities, gestational age at birth, birth weight, and diagnosed comorbidities were initially modeled but were ultimately excluded, as these caused only negligible adjustments in associations with our primary predictors and had no significant associations with outcomes. For all models, *P* values related to main hypotheses (ASD effect, ASD*Study interaction) are reported following FDR adjustment for multiple comparisons; these are reported in table S4. Where study-specific post-hoc tests were conducted, all *P* values reported reflect model-specific Bonferroni adjustment.

For classification with penalized logistic regression, a LASSO model was implemented as per Tibshirani (*34*). The objective function for this was

$$\min_{(\beta_0,\beta)} \left[ \frac{1}{N} \sum_{i=1}^{N} y_i \cdot (\beta_0 + x_i^T \beta) - \log(1 + e^{(\beta_0 + x_i^T \beta)}) \right] + \lambda \|\beta\|_1$$

A selection parameter, α, was fixed at 1 for the LASSO procedure, with outcome (ASD case or control) *y*, model intercept $\beta_0$, shrinkage parameter $\lambda$, $x_i^T \beta$ the matrix of predictor variables and associated beta estimates, and $\|\beta\|_1$ the L1 norm regularization term. The predictor variables in

the model included all RQA features derived from single-element and dual-element (cross-recurrence) analyses. The glmnet package (v2.0-13) in R was used to implement the model. Supplementary Table 5 lists the features that survived shrinkage during cross-validation of the model, and their associated parameter estimates.

For weighted quantile sum (WQS) regression, models followed the form

$$g(\mu) = \beta_0 + \beta_1 \sum_j w_i q_{ij} + z'\varphi$$

Here, $\beta_0$ indicates the model intercept, $z$' and $\varphi$ indicate a vector of covariates and associated regression parameters, and $\beta_1 \sum_j w_i q_{ij}$ reflects the regression parameter ($\beta_1$) and weighted quantile sum index ($\sum_j w_i q_{ij}$), where $w_i$ indicates the estimated weight for each predictor variable and $q_{ij}$ reflects the ranked (here, quartiles) measure per subject; the summation of these terms ($w_i q_{ij}$) provides the weighted quantile sum index. A link function, $g(\mu)$, allows the generalization of this equation to multiple distributions; for classification, as in this study, the logit link to the binomial distribution was used, i.e. $\log(\frac{\mu}{1-\mu})$, where μ is the probability of a subject diagnosed as an ASD case. All parameters, including weights, are estimated via maximal likelihood estimation, where final weights are averaged across 100 bootstrapped samples, with weights constrained to vary between 0-1, and to sum to 1.

During model training, a subset of the data are used to estimate weights for each variable, which are then used to calculate and test a WQS index in the validation dataset. The weights (training set) and parameter estimates (validation set) from these models are then applied for prediction in

the naïve data in the holdout dataset. Model fit and variable weights from WQS regression are provided in Supplementary Figure 4.

## Supplementary Results

### Population-Independent Properties of Zinc-Copper Cycles

It is possible that highly evolved zinc-copper cycles have consistent properties within a species and, therefore, would show small variances across geographic populations, similar to other physiologic set points such as normal body temperature or blood pressure. We were able to test whether copper-zinc cycles are population independent using an equivalence testing approach (*55*). This statistical method is commonly used to test for bioequivalence between the efficacy of pharmaceuticals (*55*). We found that for all three measures of zinc-copper rhythmicity, the four different populations tested were bioequivalent (fig. S3).

### Dysregulation of Other Elemental Cycles

Earlier work has shown that lead exposure is associated with autism risk (*1*), and zinc is known to be protective against the toxic effects of lead in animal models (*56*). We observed that ASD cases exhibited zinc-lead cycles that were different to their non-ASD counterparts (fig. S1 and table S1). As with the zinc-copper cycles, overall cases displayed reduced determinism ($P<0.001$) and entropy ($P<0.05$), and tended to exhibit reduced MDL ($P=0.06$), but these differences were not consistent across populations, particularly for determinism.

As an exploratory analysis, we also examined cycles of individual elements (table S2). We found that copper rhythms were elongated (higher mean diagonal length, $P=0.002$) in ASD cases and showed greater complexity (increased entropy, $P=0.001$) and determinism ($P=0.002$) in ASD

cases compared to controls. Copper rhythms in ASD cases also tended to have a smaller time gap between cycles, as indicated by a reduction in recurrence time type 2 ($P$=0.004). A reversal in these trends was evident for lithium, where cases showed rhythms that were shorter ($P$=0.04), less complex ($P$=0.049) and unstable ($P$=0.022) for lithium and had reduced determinism lead ($P$=0.022).

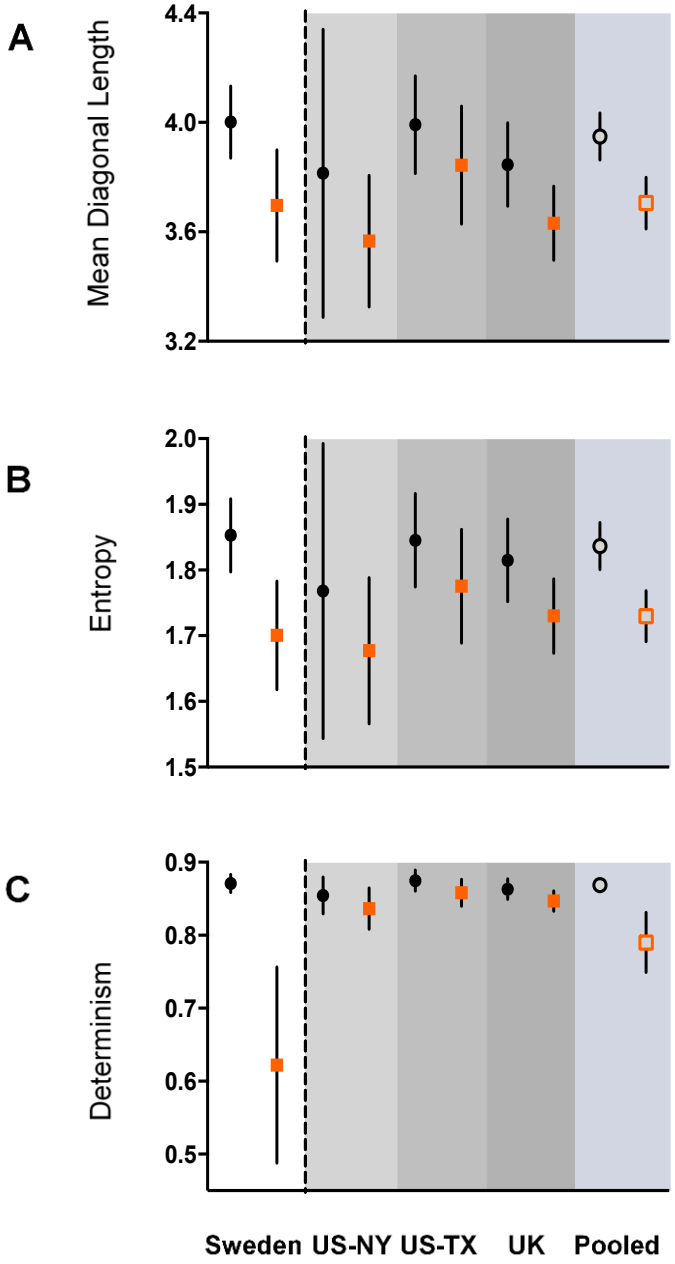


**fig. S1. Disruption of zinc-lead cycles in ASD.** Statistically non-significant reductions in mean diagonal length (**A**), entropy (**B**) and determinism (**C**) are observed in autism cases (squares) compared to controls (circles) in all study populations. Data are means +/- 95% CI. Pooled estimates generated by combining data from all studies.

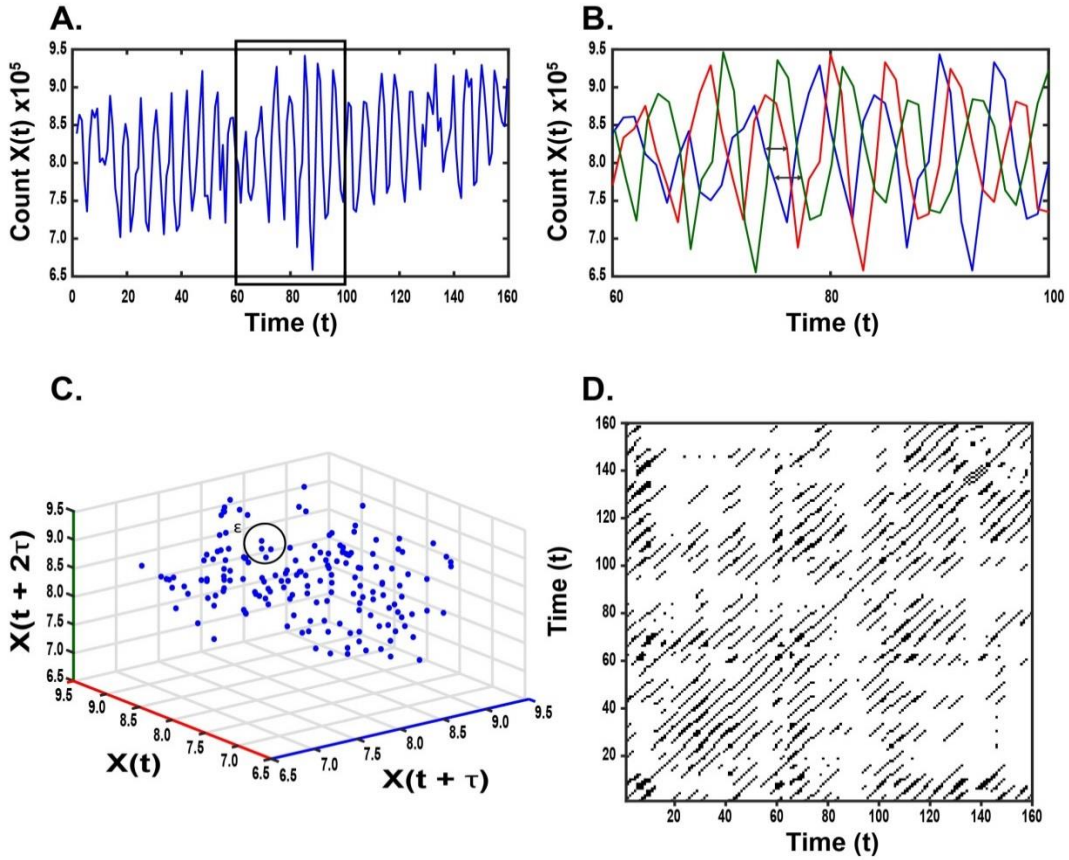


**fig. S2. Recurrence quantification analysis.** (**A**) Developmental exposure profile for Ca, expressed as counts, reflecting elemental concentrations in tooth matrices at approximately 100 sequential sampling points that reflect a time period from the second trimester to birth. (**B**) Delay embedding of the signal in (A), showing the paneled-section of the waveform. The original signal is shown in blue, with green and red lines reflecting two embeddings (time-lagged duplicates) of delay ($\tau$) 10. (**C**) Phase-portrait of the Ca time-series in (A) plotted in three-dimensions. The coordinates of a given point are determined by the value of each delay-embedded signal at that time interval. Black circle indicates an exemplar threshold ($\varepsilon$) used to construct a recurrence matrix (shown in D). (**D**) Recurrence plot of the Ca time-series. Black marks in matrix indicate time-points where the system entered a given state (within-threshold on phase-portrait); e.g., all black marks vertical of day 20 indicate times when the system returned to the state it was in at day 20. Diagonal structures in this plot reflect periods of stable cyclical orbits, while white space indicates unique values, and laminar structures (vertical/horizontal lines) reflect static states. During recurrence quantification analysis (RQA), the duration and distribution of cyclical periods are quantified. Note this figure appears also in the supplementary materials to Curtin et al. (2017).

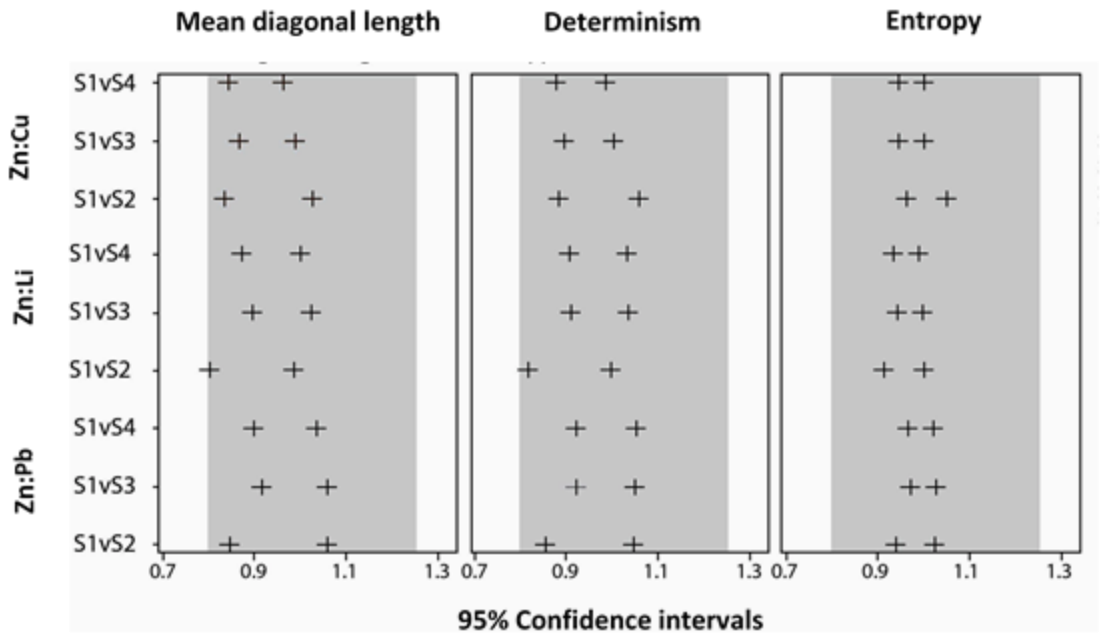


**fig. S3. Equivalence testing of control group means across studies.** Asymmetric family-wise 95% confidence intervals (based on Dunnett comparisons) on cross-recurrence measures for the ratio of study means (S1/S2, S1/S3 and S1/S4) of control groups, adjusted by sex. S1: Sweden, S2: Texas, S3: NY, and S4: UK. When the two family-wise confidence intervals are contained in the equivalence bounds of 0.8, 1.25 (shaded region), the four study means for the specified cross-recurrence are equivalent.

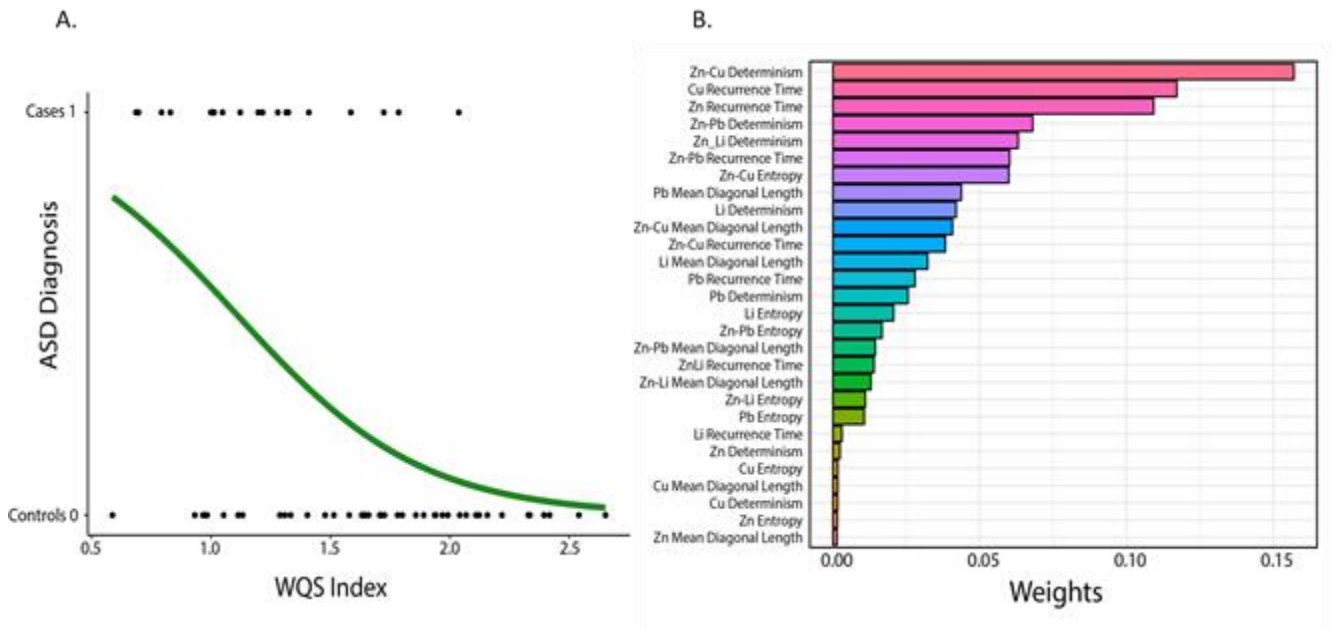


**fig. S4. Model fit and weight of variables contributing to the WQS regression model.** (**A**) Model fit in the validation dataset. Performance metrics describe classification error in the validation dataset (51% of data), from which regression parameters are estimated. (**B**) Variable weights extracted from the training dataset (34% of data).

**table S1. Results of cross-recurrence analyses.** Values in the table reflect P values from linear mixed models regressing the effect of ASD-diagnosis (Pooled column; FDR-adjusted main effect of ASD) and study-specific posthoc tests (Bonferroni-adjusted for multiple comparisons) comparing measures between ASD cases and controls within an ASD*Study interaction.

| | Measure | Sweden | US-NY | US-TX | UK | Pooled |
|---|---|---|---|---|---|---|
| **Zinc:Copper** | Determinism | 0.001 | <0.001 | 0.03 | 0.063 | <0.001 |
| | Mean Diagonal Length | <0.001 | 0.029 | 0.005 | 0.023 | <0.001 |
| | Entropy | <0.001 | 0.003 | 0.003 | 0.037 | <0.001 |
| | Recurrence Time | 1 | 0.098 | 0.63 | 1 | 0.072 |
| **Zinc:Lead** | Determinism | <0.001 | 1 | 1 | 1 | <0.001 |
| | Mean Diagonal Length | 0.25 | 1 | 1 | .43 | 0.061 |
| | Entropy | 0.067 | 1 | 1 | .46 | 0.048 |
| | Recurrence Time | 0.93 | 0.96 | 1 | .54 | 0.061 |
| **Zinc:Lithium** | Determinism | 1 | 1 | 1 | 1 | 0.73 |
| | Mean Diagonal Length | 0.69 | 1 | 1 | 1 | 0.81 |
| | Entropy | 1 | 1 | 1 | 1 | 0.65 |
| | Recurrence Time | 1 | 0.82 | 0.70 | 1 | 0.18 |

**table S2. Results of single-recurrence analyses.** Values in the table reflect P values from linear mixed models regressing the main effect of ASD-diagnosis (Pooled column; FDR-adjusted main effect of ASD) and study-specific posthoc tests (Bonferroni-adjusted for multiple comparisons) comparing measures between ASD cases and controls within an ASD*Study interaction.

| | Measure | Sweden | US-NY | US-TX | UK | Pooled |
|---|---|---|---|---|---|---|
| **Copper** | Determinism | 0.99 | 0.007 | 0.46 | 1 | 0.002 |
| | Mean Diagonal Length | 0.4 | 0.037 | 0.63 | 0.27 | 0.002 |
| | Entropy | 0.47 | 0.019 | 0.46 | 0.16 | 0.001 |
| | Recurrence Time | 0.009 | 0.058 | 0.73 | 1 | 0.004 |
| **Lithium** | Determinism | 0.038 | 0.44 | 1 | 1 | 0.022 |
| | Mean Diagonal Length | 0.059 | 1 | 0.34 | 1 | 0.04 |
| | Entropy | 0.066 | 1 | 0.67 | 1 | 0.049 |
| | Recurrence Time | 1 | 1 | 1 | 1 | 0.57 |
| **Lead** | Determinism | 1 | 1 | 0.94 | 0.012 | 0.022 |
| | Mean Diagonal Length | 0.59 | 1 | 1 | 0.24 | 0.18 |
| | Entropy | 0.64 | 1 | 1 | 0.15 | 0.13 |
| | Recurrence Time | 0.18 | 1 | 1 | 1 | 0.57 |
| **Zinc** | Determinism | 1 | 0.80 | 1 | 1 | 0.44 |
| | Mean Diagonal Length | 1 | 0.65 | 0.78 | 1 | 0.21 |
| | Entropy | 1 | 0.70 | 1 | 1 | 0.39 |
| | Recurrence Time | 0.45 | 0.34 | 1 | 1 | 0.13 |

**table S3. Laser ablation analyses of teeth.**

| **NWR-193 Laser Conditions** | | **Agilent 8800 ICP-MS Conditions** | |
|---|---|---|---|
| Wavelength (nm) | 193 | RF power (W) | 1350 |
| Helium carrier flow (L $min^{-1}$) | 0.8 | Argon carrier flow (L $min^{-1}$) | 0.6 |
| Fluence (J $cm^{-1}$) | 5.0 | Plasma gas flow (L $min^{-1}$) | 15 |
| Repetition rate (Hz) | 10 | Sample Depth (mm) | 4.0 |
| Spot size (μm) | 35 | Scan mode | Peak hopping |
| Scan speed (μm $s^{-1}$) | 35 | Integration time (ms) | 50 – 55 |

**table S4. Main effects and interactions across elemental pathways.** For each elemental pathway or cross-recurrence (column 1), the effects of ASD diagnosis or an ASD*Study interaction (rows in effect column) were tested on 4 RQA features. Test statistics and FDR-adjusted *P* values are given for each effect. FDR adjustment reflects the 56 tests done across all pathways and effects listed.

| Elemental Pathway | RQA Feature | Effect | F Value | FDR adjusted *P* |
|---|---|---|---|---|
| Zn_Cu | Determinism | ASD | 47.99 | **7.74E-07** |
| | | ASD*Study | 2.56 | 0.072 |
| Zn_Cu | Entropy | ASD | 57.3 | **1.18E-07** |
| | | ASD*Study | 1.98 | 0.140 |
| Zn_Cu | Mean_DL | ASD | 52.25 | **2.63E-07** |
| | | ASD*Study | 3.83 | **0.014** |
| Zn_Cu | RT2 | ASD | 4.64 | 0.072 |
| | | ASD*Study | 1.77 | 0.189 |
| Cu | Determinism | ASD | 15.51 | **0.002** |
| | | ASD*Study | 4.25 | **0.008** |
| Cu | Entropy | ASD | 18.2 | **0.001** |
| | | ASD*Study | 5.32 | **0.002** |
| Cu | Mean_DL | ASD | 15.6 | **0.002** |
| | | ASD*Study | 6.51 | **0.001** |
| Cu | RT2 | ASD | 12.46 | **0.004** |
| | | ASD*Study | 3.85 | **0.014** |
| Zn_Pb | Determinism | ASD | 30.21 | **1.93E-05** |
| | | ASD*Study | 12.39 | **8.64E-07** |
| Zn_Pb | Entropy | ASD | 5.84 | **0.048** |
| | | ASD*Study | 0.93 | 0.552 |
| Zn_Pb | Mean_DL | ASD | 5.03 | 0.061 |
| | | ASD*Study | 1.4 | 0.295 |
| Zn_Pb | RT2 | ASD | 5.05 | 0.061 |
| | | ASD*Study | 21.68 | **2.10E-09** |
| Pb | Determinism | ASD | 7.8 | **0.023** |
| | | ASD*Study | 2.42 | 0.076 |
| Pb | Entropy | ASD | 3.18 | 0.131 |
| | | ASD*Study | 2.65 | 0.061 |
| Pb | Mean_DL | ASD | 2.46 | 0.180 |
| | | ASD*Study | 2.99 | **0.044** |
| Pb | RT2 | ASD | 0.45 | 0.567 |
| | | ASD*Study | 0.87 | 0.567 |
| Zn_Li | Determinism | ASD | 0.15 | 0.725 |
| | | ASD*Study | 2.86 | **0.048** |
| Zn_Li | Entropy | ASD | 0.26 | 0.649 |
| | | ASD*Study | 1.43 | 0.289 |
| Zn_Li | Mean_DL | ASD | 0.06 | 0.811 |
| | | ASD*Study | 2.47 | 0.072 |
| Zn_Li | RT2 | ASD | 2.5 | 0.179 |
| | | ASD*Study | 1.3 | 0.338 |
| Li | Determinism | ASD | 7.91 | **0.022** |
| | | ASD*Study | 2.26 | 0.096 |
| Li | Entropy | ASD | 5.65 | **0.050** |
| | | ASD*Study | 2.18 | 0.106 |
| Li | Mean_DL | ASD | 6.43 | **0.040** |
| | | ASD*Study | 1.47 | 0.278 |
| Li | RT2 | ASD | 0.41 | 0.567 |
| | | ASD*Study | 0.56 | 0.772 |
| Zn | Determinism | ASD | 0.81 | 0.436 |
| | | ASD*Study | 1.68 | 0.204 |
| Zn | Entropy | ASD | 0.98 | 0.392 |
| | | ASD*Study | 4.9 | **0.003** |
| Zn | Mean_DL | ASD | 2.06 | 0.213 |
| | | ASD*Study | 5.95 | **0.001** |
| Zn | RT2 | ASD | 3.14 | 0.131 |
| | | ASD*Study | 10.5 | **5.20E-06** |

**table S5. Features preserved in the penalized logistic regression classifier.** All features included during cross-validation and prediction are listed by elemental pathway (column 1) and dynamical feature extracted via recurrence quantification analysis (RQA) or cross-recurrence quantification analysis (CRQA) (column 2). Associated beta estimates are provided in column 3, with blank values (.) indicating features zeroed during LASSO shrinkage.

| Elemental pathway | RQA Feature | Beta |
|---|---|---|
| Cu | Determinism | 2.0604 |
| Cu | Entropy | 4.0472 |
| Cu | Mean Diagonal Length | . |
| Cu | RT2 | -0.062 |
| Li | Determinism | . |
| Li | Entropy | . |
| Li | Mean Diagonal Length | -0.653 |
| Li | RT2 | . |
| Pb | Determinism | -5.623 |
| Pb | Entropy | . |
| Pb | Mean Diagonal Length | . |
| Pb | RT2 | . |
| Zn | Determinism | 14.538 |
| Zn | Entropy | . |
| Zn | Mean Diagonal Length | 0.8691 |
| Zn | RT2 | -0.023 |
| Zn_Cu | Determinism | -13.89 |
| Zn_Cu | Entropy | -1.295 |
| Zn_Cu | Mean Diagonal Length | -3.159 |
| Zn_Cu | RT2 | -0.298 |
| Zn_Li | Determinism | -0.746 |
| Zn_Li | Entropy | -0.658 |
| Zn_Li | Mean Diagonal Length | . |
| Zn_Li | RT2 | -0.169 |
| Zn_Pb | Determinism | -3.683 |
| Zn_Pb | Entropy | . |
| Zn_Pb | Mean Diagonal Length | . |
| Zn_Pb | RT2 | . |